In yet another further aspect, the present invention provides an amino acid sequence of the recombinant ScFV : GyraA protein as shown in Seq. ID # 2, which inhibits DNA gyrase from *M. smegmatis* and *M. tuberculosis*.

In yet another embodiment of the invention, said engineered single chain antibody contains an amino acid sequences which inhibit the activity of DNA gyrase from *M. smegmatis* and *M. tuberculosis*, said amino acid sequences having the Seq. ID # 3 and Seq. ID # 4 respectively.

In a further aspect, the present invention provides monoclonal antibodies, viz. MSGyrA:C3 and MSGyrA:H11, which inhibits DNA gyrase from fluoroquinolone resistant *M. smegmatis* and *M. tuberculosis*.

In yet another further aspect, the present invention provides hybridoma cell lines C3B3 and H11E1, which secrete the monoclonal antibodies, MSGyrA:C3 and MSGyrA:H11, which also inhibits DNA gyrase from *M. smegmatis* and *M. tuberculosis*.

The monoclonal antibody (mAb) described in this invention has been generated against GyrA subunit of *M. smegmatis* DNA gyrase. The mAb cross reacts with GyrA subunit from fast and slow growing mycobacteria (U. H. Manjunatha et. al., *Eur. J. Biochem.*, **2001**, 268, 2038-2046). The invention describes the inhibition of DNA supercoiling activity catalyzed by *M. smegmatis* and *M. tuberculosis* DNA gyrases by full-length mAb and its Fab and single chain antibody (scFv) fragments. The present invention also describes inhibition of DNA gyrase activity by peptides derived from scFv. The invention also deals with novel mechanism of DNA gyrase inhibition is distinct from that of other known DNA gyrase inhibitors.

DESCRIPTION OF THE FIGURES

Figure 1A : Specificity of interaction of mAb.

Figure 1B : Effect of mAbs on mycobacterial DNA gyrase supercoiling activity.

Figure 2A : Effect of MsGyrA:C3 on DNA binding.

Figure 2B : Effect of MsGyrA:C3 on DNA cleavage.

Figure 2C : Effect of MsGyrA:C3 on ATP hydrolysis.

Figure 2D : Effect of MsGyrA:C3 on ATP independent DNA relaxation reaction of mycobacterial DNA gyrase.

Figure 3A and 3B : Effect of MsGyrA:C3 on quinolone resistant M. *smegmatis* DNA gyrase.

inhibited at 3 μg/ml and 6 μg/ml concentrations of MsGyrA:C3 for quinolone sensitive (D^S) and quinolone resistant (D^R) enzymes respectively (Figure 3B). The twofold difference in the mAb concentration between D^S and D^R enzymes is attributed to reduced specific activity of D^R enzyme. DNA gyrase from ofloxacin resistant, highly virulent clinical isolate of *M. tuberculosis* (ICC-222) was also assayed for the effect of mAb. The purified enzyme has an IC_{50} of ~10 μg/ml for ciprofloxacin, where as the MsGyrA:C3 inhibited DNA gyrase supercoiling activity at 3.0 μg/ml, similar to that of *M. smegmatis* enzyme (Figure 3C). The absence of cross-resistance essentially emphasizes the mode of action of mAb to be distinct to that of quinolones. Similar to MsGyrA:C3, MsGyrA:H11 also inhibited ciprofloxacin resistant *M. smegmatis* DNA gyrase (Figure 3D). These data confirm the novel inhibition mechanism of gyrase by mAb. Absence of cross-resistance to fluoroquinolone resistant DNA gyrase by mAb, warrants the study of MsGyrA:C3 further as it could aid in countering the drug resistance problem.

E. Cloning, sequencing and expression of a DNA sequence coding for neutralizing antibody gene and design of bioactive peptides

This example describes the cloning and expression of a nucleic acid sequence coding for a DNA gyrase neutralizing monoclonal antibody, MsGyrA:C3. Based on the inhibition of gyrase by scFv:GyrA and utilizing sequence of the antibody, bioactive peptides were designed and their inhibition of mycobacterial DNA gyrase was tested.

E1 : ***Cell culture and Isolation of RNA:***

Total RNA was isolated from the actively secreting mAb:C3 hybridoma cell line. Briefly, confluent hybridoma cells ($3X10^8$) were washed with ice cold IMDM medium and total RNA was extracted using TRIzol reagent (Life technologies Inc). RNA was purified using RNeasy QUIAGEN as per the manufacturer's protocol. The quality of RNA was confirmed by electrophoresis in a 1% formaldehyde agarose gel.

E2 : ***First-strand cDNA synthesis:***

The first-strand cDNA was synthesized from total RNA using the reverse transcription reaction (RT). For annealing, 5 μg of total RNA was incubated with 0.2 μg/ml of random hexamer oligonucleotide in a 10 μl reaction volume at 70°C for 5 minutes, followed by immediate chilling on ice. The annealed mix was incubated with 1 mM dNTP and 20 Units of Moloney Murine Leukemia Virus reverse transcriptase, (M-

Claims

1. An engineered single chain antibody, which inhibits the activity of DNA gyrase from *M. smegmatis* and *M. tuberculosis*.
2. The engineered single chain antibody as claimed in claim 1 wherein it contains amino acid sequences for inhibiting the activity of DNA gyrase from *M. smegmatis and M. tuberculosis* said amino acid sequences having the Seq. ID # 3 and 4 respectively.
3. An engineered single chain antibody as claimed in Claim 1 wherein said antibody has a nucleotide sequence shown in Seq. ID # 1.
4. An engineered single chain antibody as claimed in Claim 1 wherein said antibody has an amino acid sequence shown in Seq. ID # 2.
5. A peptide having an amino acid sequence as shown in Seq. ID # 2.
6. A process for the preparation of an engineered single chain antibody which inhibits the activity of DNA gyrase from *M. smegmatis* and *M. tuberculosis*, said process comprising preparing complimentary DNA (cDNA) from the corresponding hybridoma cell lines which secretes monoclonal antibody, amplifying from said cDNA, DNA fragments encoding variable heavy chain region and light regions of said monoclonal antibody, fusing said variable heavy chain region and light regions of said DNA fragments, cloning said fused DNA fragment in a plasmid, transforming said plasmid into *E. Coli* host strain, inducing said transformed cells to express said engineered single chain antibody and purifying said engineered single chain antibody from the induced cell lysate.
7. Monoclonal antibodies, which inhibit DNA gyrase from fluoroquinolone resistant *M. smegmatis* and *M. tuberculosis*.
8. A plasmid which inhibits the activity of DNA gyrase from *M. smegmatis* and *M. tuberculosis*, said plasmid being characterised in that it encodes an engineered single chain antibody containing amino acid sequences for inhibiting the activity of DNA gyrase from *M. smegmatis and M. tuberculosis* said amino acid sequences being as shown in Seq. ID # 3 and 4 respectively.